Cyanidin-3-glucoside enhances mitochondrial function and biogenesis in a human hepatocyte cell line.

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Abstract of thesis

(Purpose)

Mitochondrial dysfunction has been identified as one of the primary factors contributing to liver diseases. The author, Mr. Rashad Mogalli, took note of mitochondrial biogenesis pathways and its potential therapeutic target molecules for the amelioration of hepatocyte dysfunction and liver disease. Research on natural pharmacological agents that ameliorate liver diseases has intensified over the last two decades. Cyanidin-3-glucoside (Cy3g), a dietary flavonoid compound extracted from a wide variety of fruits and vegetables, reportedly has several beneficial health effects. In this study, he used an adult human hepatoma cell line (HuH7) to investigate the effects of the Cy3g polyphenolic compound on mitochondrial function and biogenesis *in vitro*.

(Materials and Methods)

Cy3g (98% HPLC Purity) was purchased from Tokiwa Phytochemical Co., Ltd. Japan. The well-differentiated human hepatocellular carcinoma HuH7 cell line was purchased from the National Institutes of Biomedical Innovation Health and Nutrition JCRB Bank (JCRB No. JCRB0403, Tokyo, Japan). Using the agent and cell line, the author performed MTT assay and Guava Count assay

to assess viable and apoptotic cells. To evaluate the mitochondrial function, ATP assay and mitochondrial membrane potential assay were performed. Furthermore, mRNA expression levels of mitochondrial function-related genes were measured by RT-PCR; peroxisome proliferator-activated receptor -1 alpha (PGC-1α), sirtuin 1 (SIRT1), mitochondrial transcription factor A (TFAM), nuclear respiratory factor-1 (NRF1), carnitine palmitoyltransferase 1 beta (CPT-1β), and phosphofructokinase 1 (PFK-1) were evaluated.

(Results)

The author found an increase in intracellular mitochondrial reductase levels after treatment with Cy3g, but not cytotoxic activity. In addition, mitochondrial membrane potential and ATP production were increased following Cy3g treatment. The author further revealed that Cy3g treatment also resulted in a dose- and time-dependent upregulation of the gene expression of PGC-1α, a transcription factor considered a master regulator of mitochondrial biogenesis and metabolism. Additionally, the expression of SIRT1, which plays a key role in deacetylating PGC-1α, was increased in a dose- and time-dependent manner. Next, the author studied the downstream gene expression of *PGC-1α* after Cy3g treatment, and confirmed *NRF1* and *TFAM* were up-regulated. He found that this pathway contributed to amelioration of mitochondrial dysfunction of hepatocyte in Cy3g treatment.

(Discussion)

The author first mentions about Cy3g used in his study in the discussion section. Several studies have reported the beneficial effects of a variety of natural compounds, such as resveratrol, quercetin and catechin on health, and among anthocyanin compounds, Cy3g, a phenol pigment that belongs to the flavonoid family, has been shown to have beneficial effects in several *in vitro* and in clinical trials. Cy3g enhances skeletal muscle mitochondrial biogenesis by upregulating PGC-1α levels. PGC-1α is reportedly an essential factor for upregulating hepatic metabolism and is key for overall liver metabolism. These pre-existing reports seem to be a reason why the author used this compound, Cy3g, in his experiments, and it is considered feasible. His study examined the ability of Cy3g to increase mitochondrial function and biogenesis in HuH7 and elucidated the underlying mechanism.

The author found that Cy3g induced PGC-1α. The induction of *PGC-1α* gene expression was associated with an increased *SIRT1* gene expression. Increased expression of PGC-1α-coactivated downstream genes, such as *NRF1*, which encodes respiratory chain subunits and other proteins necessary for mitochondrial function, was observed. Moreover, Cy3g increased the gene expression of *TFAM*, a nuclear-encoded transcription factor that plays a key role in mitochondrial DNA replication and transcription and is regulated by NRF1. Cy3g also increased *CPT-1β* gene expression, which is located within the mitochondrial outer membrane and is considered the rate-limiting enzyme of mitochondrial β-oxidation as CPT-1β controls the mitochondrial uptake of long chain acyl-CoA fatty acids. Cy3g also increased *PFK*-gene expression, an important regulator of glycolysis.

The author here speculates a potential protective effect of Cy3g for hepatocyte from metabolic stress, and discusses with the reported literatures as follows. Currently, lifestyle modification and caloric restriction are the only treatments for nonalcoholic fatty liver diseases. Some polyphenol compounds, such as resveratrol, have shown calorie restriction-mimicking effects in mammalian diseases and can ameliorate liver fat accumulation in high-fat diet mouse models, mostly due to the activation of metabolism-sensing signaling systems.

While several pathways control mitochondrial function, biogenesis and free fatty acid oxidation, a recently identified member of peroxisome proliferator-activated receptor gamma coactivator family, PGC-1α, serves as a major regulator of the nuclear receptors that control metabolic pathways and is expressed in tissues with high oxidative capacity. Hepatocytes extracted from PGC-1α-deficient mice exhibit reduced mitochondrial respiration rates, indicating a reduced hepatic fatty acid oxidation capacity. SIRT1 coexists with the transcription factor PGC-1α and plays an important role in PGC-1α activation via deacetylation. SIRT1 and PGC-1α signaling is important in the protection of *in vitro* hepatocyte models against mitochondrial oxidative stress. Furthermore, pharmacological activation of SIRT1 by polyphenol in HepG2 cells protected against FAS induction and lipid accumulation. Several studies have revealed the crucial role of sirtuins generally and SIRT1 specifically in liver diseases. SIRT1, an NAD+ dependent protein deacetylase, is an important regulator of energy homeostasis, enhanced mitochondrial metabolism, antioxidative protection, lipid catabolism and glucose homeostasis. Both *in vitro* and *in vivo* models of SIRT1 deficiency have shown a tendency for increased lipid accumulation in the liver and downregulation of *de novo* hepatic lipid synthesis transcription factors, such as sterol regulatory element binding protein-1c and carbohydrate response element binding protein.

Finally, the author discusses therapeutic potential of Cy3g, and of targeted-molecules on its signal transduction pathway. Pathways that control mitochondrial biogenesis have been studied extensively to identify future therapeutic approaches to treat the mitochondrial dysfunction that leads to various liver and metabolic diseases. His experiments showed that in a human-derived hepatocyte HuH7, Cy3g is a potent activator of the SIRT1 and PGC-1α signaling pathways, inducing mitochondrial biogenesis and function and triggering an increase in *PGC-1α* downstream genes, and these effects are dose- and time-dependent. Therefore, he concluded that this compound should be considered a therapeutic or preventive approach for diseases caused by hepatic cell mitochondrial dysfunction.

Abstract of assessment result

(General Comments)

In this paper, the author evaluated the effects of Cy3g on human liver mitochondrial function and biogenesis, using a human-derived hepatocyte HuH7. Cy3g is a dietary flavonoid compound extracted from a wide variety of fruits and vegetables. It is easily available in daily life. He studied the effects with a variety of methods, and suggested that Cy3g enhanced mitochondrial function and biogenesis, and had potential as a hepatoprotective therapeutic agent. Furthermore, he examined the intracellular signal pathway on which Cy3g worked, and confirmed the contribution of SIRT1 and PGC-1α signaling. PGC-1α has been reported as an inhibitor of mitochondrial oxidative stress and modulator of fatty acids. These findings are considered to be important for the future clinical development of therapeutics in patients who have hepatic dysfunction due to fatty liver.

(Assessment)

The final examination committee conducted a meeting as a final examination on December 26, 2018. The applicant provided an overview of dissertation, addressed questions and comments

raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

(Conclusion)

The final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Medical Sciences.